



## Multiphoton Imaging: A New Generation of Fiber Lasers Combine Cutting Edge Performance with 24/7 Industrial Reliability

Power-scalable ytterbium fiber technology, combined with HALT/HASS design and testing protocols, has yielded a comprehensive family of tunable and fixed wavelength ultrafast laser sources optimized to address the most recent trends in multiphoton imaging and optogenetic photoactivation. Fidelity, Discovery and Monaco lasers all deliver cutting-edge performance with industrial-grade reliability, emblematic characteristics of our design and manufacturing approach summarized as The Industrial Revolution in Ultrafast Science. They provide higher power, shorter pulsewidths and a broader wavelength range – particularly for longer wavelengths – all the while delivering 24/7 laser uptime and, hence, maximum data output.

### The Need for Higher Power and Longer Wavelengths

Several trends in multiphoton microscopy applications, particularly in neuroscience, are currently driving the demand for ultrafast lasers that deliver higher output powers and longer wavelengths. First is the push for deeper imaging in live tissue and animals, which in turn requires more power – to counteract absorption and scattering losses – and longer laser wavelengths – to minimize scattering losses. Second, higher power is also needed for faster and wider imaging – to simultaneously image many neurons and follow their activity in real time. Third, in the newer area of optogenetics and all-optical physiology, two-photon activation requires considerable power and longer wavelengths to minimize crosstalk with imaging and signaling probes co-expressed in the same sample. Finally, new three-photon experiments require even longer wavelengths, up to 1.7 micrometers to maximize functional imaging depth in model animals.

There is thus a need for tunable and turnkey sources of femtosecond laser pulses in the 900 – 1,300 nm wavelength range at multi-watt power levels, and even higher power for some optogenetics studies. One-box lasers based on Titanium:Sapphire (Ti:S), such as the Chameleon Vision series, revolutionized multiphoton imaging and continue to be workhorses in many imaging studies but their power decreases considerably in proximity of their long-wavelength limit (1,000 – 1,080 nm); wavelengths to 1,300 nm are readily available with OPO accessories, but their output powers are typically less than 1 watt.



To avoid these power and wavelength limitations, laser manufacturers started to look for other active laser materials that could better match these requirements.

## Ytterbium Doped Fiber

Ytterbium doped materials have long been seen as potential alternative gain materials in the laser community because of several positive attributes. Most importantly, they can be directly pumped with cost-effective laser diode components, eliminating altogether the need for a solid-state green pump laser. Nevertheless ytterbium-doped crystals present the laser designer with thermal management challenges similar to the ones limiting commercial Ti:S lasers to less than 5 watts. Low laser gain and the three-level physics of ytterbium active media require substantial pump powers (and heat management) thus preventing a simple route to deliver reliable higher power performance from bulk crystals of this material.



*Figure 1. Fidelity-2 is a compact, sealed industrial-grade laser that provides over 2 watts of output power at 1070 nm with a pulsewidth < 55fs.*

Recognizing the primary importance of a power-scalable roadmap, Coherent has invested many years of R&D focusing on ytterbium-doped fibers, where the cooling challenge is avoided – thanks to the totally different geometries of the medium and pumping scheme, including a more efficient and distributed absorption of the pump power. In addition to power scalability, the use of fiber enables laser architectures that can deliver higher stability, better beam quality and superior reliability. However, obtaining the optimum combination (for multiphoton imaging) of short pulsewidth and high overall power proved to be more challenging. The gain bandwidth of ytterbium-doped glass is wide enough to deliver transform limit pulses of < 150 fsec, which is a good match for most multiphoton imaging and photoactivation studies. But

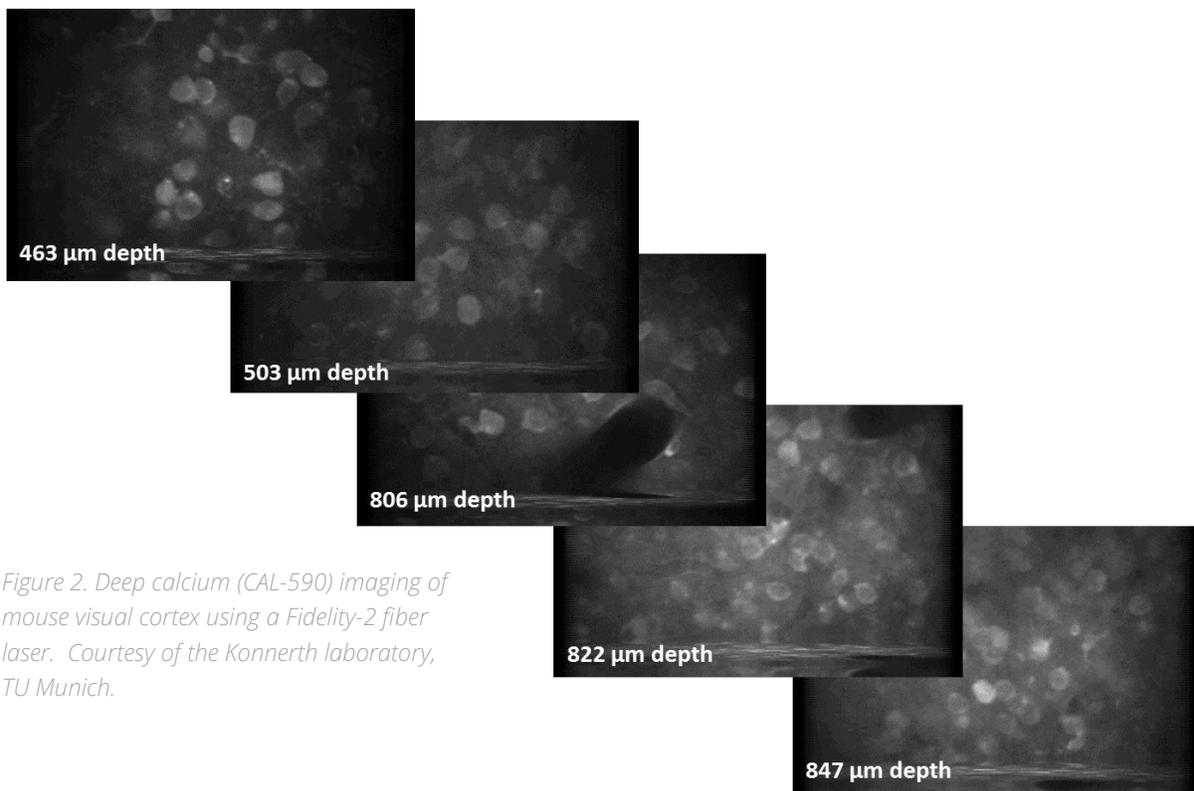


there is a trade-off between gain amplification and pulse broadening that had limited earlier attempts at high peak power lasers using ytterbium-doped fiber.

To circumvent this trade-off, Coherent engineers developed a proprietary solution that has enabled the development of lasers providing high peak power, short pulses, and other advantages needed for all the experimental variations of imaging and photoactivation employed in neuroscience. Just as important, the inherently rugged stability of fiber architecture means that these lasers can deliver their cutting-edge scientific performance with 24/7 reliability that is the cornerstone of "The Industrial Revolution in Ultrafast Science."

### Fidelity-2: Compact and Simple

The most compact of these lasers is the Fidelity-2, a single-stage fiber oscillator that provides over 2 watts of power with a pulsewidth of <55 fs at a fixed 1070 nm wavelength. Maintaining the short pulses/high peak power of this laser on the sample is made easy by the built-in pre-compensation that minimizes pulse duration at the sample, even after complex beam delivery systems. This small, sealed laser is ideal for photoactivation of the latest red-shifted opsins such as Chrimson, ReaCh or C1V1. Additionally, the Fidelity-2 is an ideal source for imaging red-shifted Genetically Encoded  $\text{Ca}^{2+}$  Indicators (GECI) such as the RCaMP family.



*Figure 2. Deep calcium (CAL-590) imaging of mouse visual cortex using a Fidelity-2 fiber laser. Courtesy of the Konnerth laboratory, TU Munich.*



### **Fidelity-HP: Up to 18 Watts at 1040 nm**

Some applications – e.g., photoactivation of large populations of neurons – require over 10 watts of femtosecond power. This need is met by the Fidelity-HP series, which uses a master oscillator power amplifier (MOPA) architecture in a single box. Fiber based MOPAs are well-proven in other Coherent lasers for numerous demanding industrial applications. Fidelity-HP leverages our expertise in chirped pulse amplifiers (CPA) to ensure unmatched performance in terms of spectral bandwidth and noise, as well as the world’s highest peak power in the near-IR imaging window. Specifically, Fidelity-HP lasers deliver pulsewidths of <140 fs at 1040 nm and up to 18 watt of output power offering also a clear roadmap to even higher power.

### **Chameleon Discovery: Compact & Tunable**

The Chameleon family of lasers is well-established as the leading choice among tunable ultrafast lasers designed for multiphoton microscopy. The latest Chameleon Discovery combines the proven Fidelity ytterbium fiber amplifier with a tunable optical parametric oscillator (OPO), all enclosed in a single sealed box. Chameleon Discovery delivers 1-2 watts of output tunable across a wide range of near-infrared wavelengths (680 nm – 1,300 nm). Moreover, part of the fixed (1,040 nm) wavelength beam of the ytterbium fiber laser is directly available as a second output with the same beam quality and short pulsewidth as Fidelity HP, as required in multi-wavelength experiments such as all-optical physiology and Raman techniques (CARS and SRS).

### **Monaco/Opera-F: Multiple Watts of Broad Wavelength Tunability**

Even higher powers and energy/pulse are available by using a Monaco laser and its Opera-F tunable optical parametric amplifier (OPA). Monaco is an ytterbium fiber-based MOPA that delivers up to 40 watts at a fixed 1,035 nm wavelength with adjustable repetition rate from single shot to 50 MHz. The Opera-F is a new type of OPA that incorporates both a non-collinear stage to enable pulsewidths as short as 50 fs, and a collinear stage that ensures broad wavelength tuning. When pumped by Monaco operated at 1 MHz repetition rate, the Opera-F delivers up to 3 watts of power across the near-IR and well beyond the limit of the optical imaging window at 1,600 nm. The high power at 1035 nm is ideal for forward-looking photoactivation applications targeting tens to hundreds of neurons, while the tunable output and repetition rate of Opera-F makes it ideal for advanced 3-photon imaging.

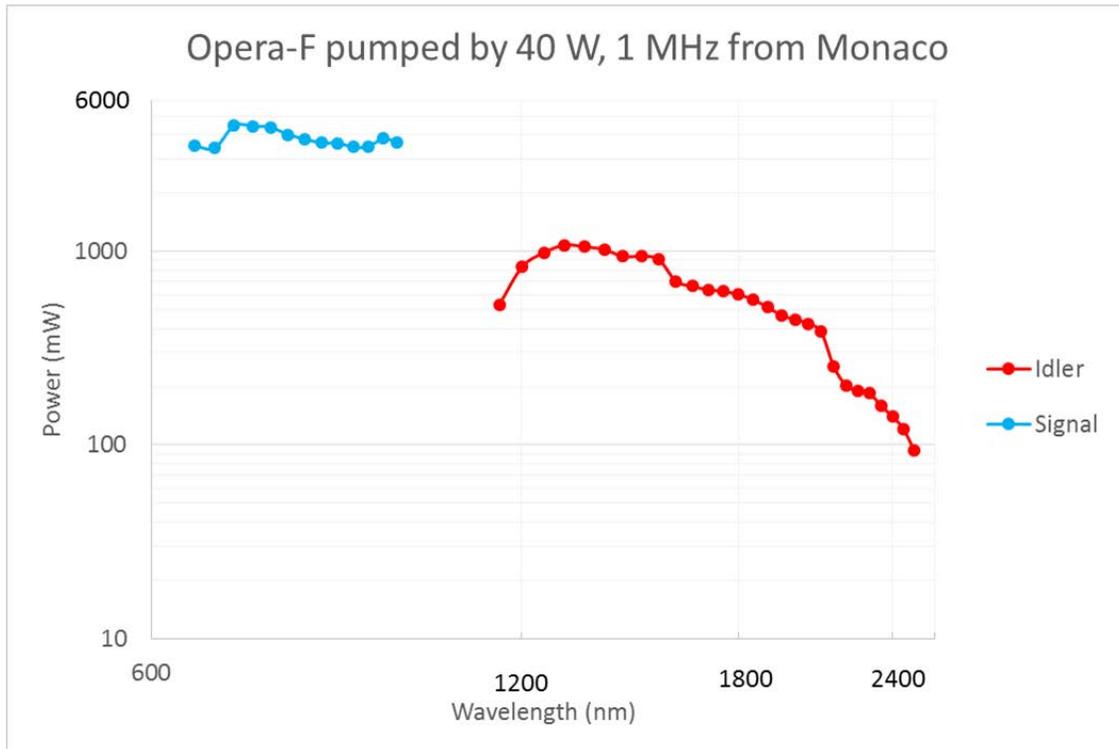


Figure 3. The combination of the Monaco laser and the Opera-F tunable optical parametric amplifier (OPA) provides several watts of power across the near-IR.

### The Advantages of Yb Fiber Lasers

This next generation of ytterbium fiber-based lasers delivers a unique combination of important advantages for multiphoton microscopy applications, particularly for those involving optogenetics.

**Industrial 24/7 reliability.** Laser reliability and beam quality/stability are more important than ever in neuroscience as model animals with new, sophisticated and sometimes time-sensitive genetic expression are increasingly adopted. Moreover, repetitive imaging over longer periods – “chronic imaging” – requires stable, repeatable output for experimental windows spanning weeks or even months.

Fiber laser technology has long offered (but not always delivered) the promise of superior reliability and stability, compared to systems based on free space optics and separate opto-mechanical components. The design and manufacturing approach that constitutes “The Industrial Revolution in Ultrafast Science” has been implemented to deliver this superior reliability. This methodology applies decades of experience in building industrial lasers for



demanding operating conditions, as well as the laser industry's first rigorous use of HALT/HASS design protocols and test chambers. HALT stands for Highly Accelerated Life Testing and HASS stands for Highly Accelerated Stress Screening. Basically, HALT is used in the design phase to stress-test components and lasers beyond their failure limits and to thereby identify and eliminate possible weaknesses. HASS is used in the production phase and identifies manufacturing errors and unexpected deviations in automated procedures, material or tooling. As an example, the HALT protocol for Discovery included dropping the laser from a 2 meter height in its normal shipping packaging. (This is twice the height of the normal tailgate of a shipping truck.) To pass this extreme test the laser had to show no measureable change in performance.

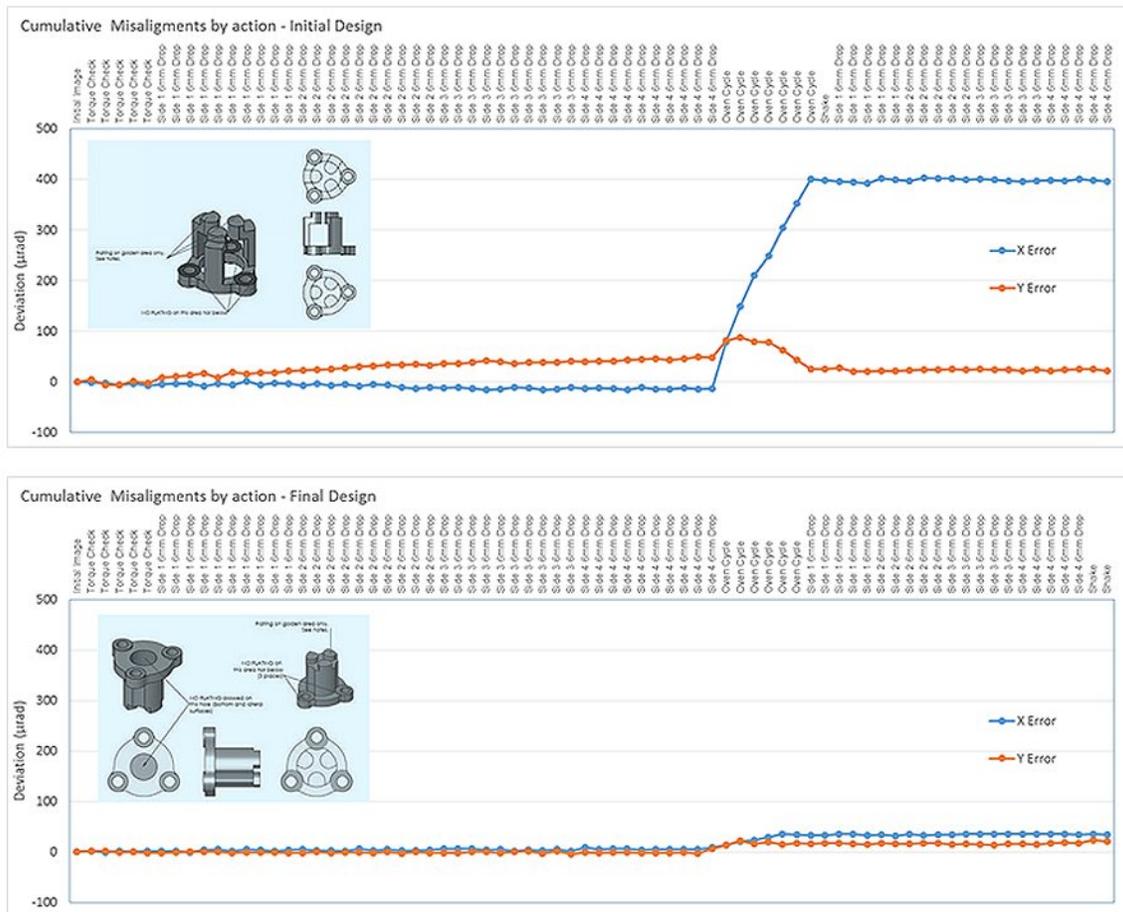


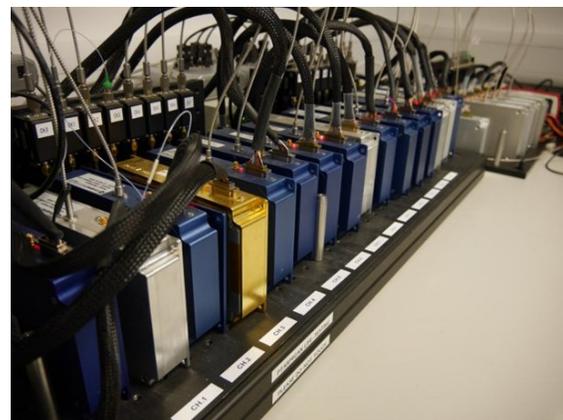
Figure 4. Upper graph - HALT testing of optical base mechanical units showed that extreme thermal cycling could cause undesirable misalignments, particularly in the X axis. Lower graph - the redesigned base unit reduced cumulative misalignment by an order of magnitude.



*Figure 5. A Chameleon Discovery laser is dropped 2m from a forklift during the exhaustive HALT design phase*

**Low cost of ownership.** In the world of industrial lasers, it is well understood that the true cost of ownership for a laser includes many factors beyond just the initial purchase cost. In scientific applications, it is more appropriate to define a “cost of data” (meaning publishable results that contribute to the success of a research team). This is harder to quantify, but with present day funding and competitive pressure, it is more important than ever before. When factors like maintenance costs, wasted student or faculty time, missed publications or funding deadlines are aggregated under the umbrella of productivity, these new ytterbium fiber laser engines deliver the lowest cost of data of any multiphoton lasers.

**Ease of Use.** These new lasers are primarily intended for use in challenging multiphoton microscopy applications where the focus of the research effort should be on the experiment itself with minimal laser expertise required. They take full advantage of their streamlined design platforms (diode-pumped fiber lasers) to attain unprecedented operational simplicity and reliability. As a result, they should provide years of operation with no scheduled maintenance or opto-mechanical adjustments to the laser head. For this reason, they are housed in sealed boxes where all tuning (in the case of Discovery) and other controls are accomplished through a simple software interface. These lasers do not require any kind of periodic component adjustment on the part of users or service personnel.



*Figure 6. Lifetesting rack for ultrafast seed laser modules. Many systems now exceed 60,000 hours continuous runtime, demonstrating the ability of fiber lasers to deliver long lifetime and low cost of ownership.*



**Power Scalability.** In optogenetics, a state of the art in-vivo experiment might involve simultaneously studying (activating and imaging) a few tens of neurons in a mouse cortex. However scientists are already looking to expand these studies to hundreds and possibly thousands of neurons in experiments targeted at unraveling the detailed functionality of the mammalian brain. Addressing larger neuron populations require increased laser power. Ytterbium fiber is the only ultrafast laser gain medium that already offers a clear unobstructed path to higher output power.

**Superior Beam Quality.** Multiphoton excitation requires a TEM<sub>00</sub> circular beam with a smooth profile in order to enable tight focus to a small beam waist. Bulk active media tunable lasers have complex resonator geometries and thermally-induced effects that the designer has to carefully mitigate in order to get an ideal output beam; on the contrary, ultrafast fiber lasers produce a naturally round and high quality beam with minimum need for manipulation other than chirp management.

**Optimized Pulsewidths.** Multiphoton microscopy relies on short pulses to better exploit the non-linear nature of the two- (or three-) photon excitation process, so that in an ideal world, the shortest possible pulses at constant average power would produce the highest fluorescent signal (or neuron activation). In following these considerations, Coherent ytterbium fiber lasers set industry standards for the shortest available pulsewidths in their power class. Several models also include an integrated pre-compensation mechanism to offset pulse broadening due to group velocity dispersion (GVD) in downstream optics, particularly acousto-optic modulators. This can be used to ensure the shortest possible pulsewidth at the sample. Interestingly, although multiphoton imaging has a history spanning a quarter of a century, there is still a debate about the optimum pulsewidth, particularly for deeper imaging of live tissue. Shorter pulsewidths definitely improve image brightness under many circumstances but may also increase the potential for photodamage, or photobleaching. The level of pre-compensation in Coherent lasers is easily varied through the laser control software suite, enabling a user to adjust the pulsewidth at the sample over a wide range, in order to address every different set of experimental conditions.

## Summary

Cutting edge applications in multiphoton imaging require cutting edge laser performance: higher power, longer wavelengths, short pulsewidths, and broad tunability. A new generation of turnkey femtosecond laser sources based on ytterbium fiber delivers state of the art performance in all these areas, while also defining a new reliability paradigm in terms of throughput and lower cost of data.



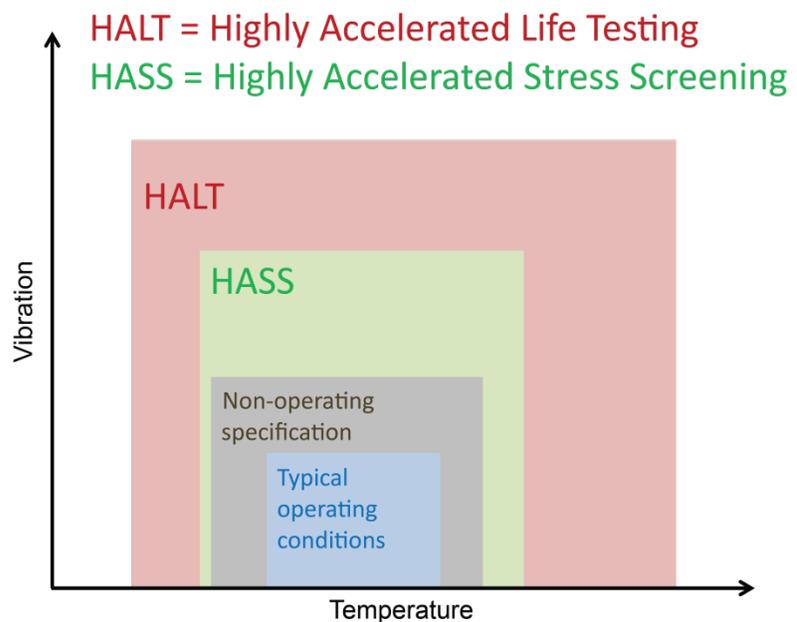
These new fiber lasers provide

- Unmatched Industrial HALT/HASS Reliability: 24/7 data throughput
- Ease of use: push button control, closed box packaging
- Low cost of ownership: superior reliability for lowest cost of data
- Low maintenance and no user alignment necessary
- Power Scalability: From watts to tens of watts
- Excellent beam quality from the fiber output: fewer aperture losses in the optical system
- Short 100 fs class pulsewidths: high peak power for brighter functional images and better stimulation

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### The Industrial Revolution In Ultrafast Science

Over the last five years, Coherent has combined decades of experience supporting 24/7 industrial laser applications with rigorous methods like HALT/HASS that are transforming product reliability from an “art” based on individual experience and assumptions into a science: experiments, results, analysis, conclusion, next action. Here we use extreme testing conditions, even as far as deliberately and repeatedly overheating, smashing and dropping, to push lasers and components to failure. These test results enable us to eliminate any inherent weaknesses in components, sub-systems and entire lasers. Iterative cycles of HALT result in a final product design that will then reliably withstand stresses beyond its specified level. HASS



*HASS involves subjecting lasers and amplifiers to stresses beyond their non-operating specification limits. HALT is used in the design and prototype phases to push components and systems to even higher limits, often to intentional failure.*



similarly identifies any potential issues during manufacturing and final assembly. HALT/HASS protocols need to be embedded in the design and manufacturing process of the laser and its subassemblies. In this respect they fundamentally differ from traditional qualification tests performed at a contractor location after the product design has been already finalized. The pervasiveness of HALT/HASS tests require the test chamber to be located where design and manufacturing functions reside, at a substantial capital cost. In addition HALT/HASS - unlike qualified “shake and bake” tests - is performed with equipment able to change the ambient temperature by 100° C in less than 2 minutes and induce vibration over broad range of frequencies, on a 5-axis configuration, to best simulate the harshest combination of adverse environmental conditions. Coherent is proud to be the first manufacturer of scientific lasers to own and consistently use independently certified HALT/HASS testing equipment for all of its current generation ultrafast products.